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THE EFFECT OF CERTAIN NATURALLY OCCURRING COMPOUNDS ON THE ACTIVITY OF RAT LUNG GLUCOSE-6-PHOSPHATE DEHYDROGENASE, GLUTATHIONE REDUCTASE AND GLUTATHIONE PEROXIDASE

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Summary

Nicotine and a number of phenolic compounds known to be components of either cigarette smoke, or tobacco, or both, were tested as possible effectors of the activities of glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase in rat lung homogenates. At a concentration of 0.4 mM, five of these compounds inhibited (up to 90% in some cases) all three of the above enzymes. Other workers have previously proposed that these same three enzymes comprise a protective mechanism in lung against damage by lipid peroxidation.

Introduction

Glutathione has been shown to reduce potentially damaging lipid peroxides in rat liver homogenates (1). Glutathione peroxidase catalyzed the involved reaction (2). DeLucia et al. (3) have reported that oxidative damage to sulfhydryl (SH) groups in rat lung by prolonged exposure to ozone is counteracted by an increase in the activity of glucose-6-phosphate (G6P) dehydrogenase and a

corresponding increase in the levels of reduced nicotinamide adenine dinucleotide phosphate (NADPH). Chow and Tappel (4) have shown that the activities of glutathione peroxidase, glutathione reductase and G6P dehydrogenase are increased significantly in lung homogenates of rats exposed to ozone. They proposed that these enzymes may comprise a protective mechanism to detoxify lipid peroxides resulting from exposure to ozone and other air oxidants. In the present paper, we report the effect of certain individual compounds present in cigarette smoke, or tobacco, or both, upon the activity of the three enzymes included in the above protective mechanism against lipid peroxidation.

Methods and Materials

Enzyme preparation: Lung homogenates were prepared according to a modification of the procedure of Chow and Tappel (4). Mature rats which had been fed a normal diet were sacrificed. Lungs were removed immediately and washed in isotonic KCl. A 20% suspension of the rat lungs in 0.25 M sucrose containing 1 mM EDTA was homogenized at 6,000 r.p.m. for 10 min. in a Sorvall Omnimixer. The homogenate was centrifuged first at 100 x g for 10 min. and then at 105,000 x g for 90 min. to yield a clear red supernatant used for enzyme assays.

Enzyme assays: The assay procedure for glutathione reductase was based upon that of Racker (5). The assay solution contained 0.05 M phosphate buffer (pH 7.6), 0.1 M NADPH, 0.1% BSA and 0.2% oxidized glutathione in a total volume of 3.0 ml. The enzyme reaction was initiated by the addition of 0.1

ml of the enzyme preparation.

Glucose-6-phosphate dehydrogenase activity was determined by the method of Brown and Wray (6), with the exception that Tris buffer (pH 7.5) was utilized according to the procedure reported for erythrocyte G6P dehydrogenase (7). The assay solution contained 0.02 M Tris-HCl buffer (pH 7.5), 0.3 mM $NADP^+$, 5 mM D-glucose-6-phosphate and 5 mM $MgCl_2$ in a total volume of 3.0 ml. The reaction was initiated by addition of 0.2 or 0.3 ml of the enzyme preparation.

The assay procedure for glutathione peroxidase was that of Paglia and Valentine (8). Commercial glutathione reductase (Sigma) was used in this assay. All effector compounds were prepared in 2% ethanol. An equivalent volume of 2% ethanol was added in control assays.

Results

The effect upon the activities of rat lung glutathione peroxidase, glutathione reductase and G6P dehydrogenase of nicotine and certain phenolic compounds known to be components of either cigarette smoke, or tobacco, or both, is shown in Table I. At a concentration of 0.4 mM, five of these phenolic compounds, namely scopoletin, scopolin, esculetin, esculin and chlorogenic acid, markedly inhibited the activity of glutathione reductase and of G6P dehydrogenase and also substantially inhibited the glutathione peroxidase assay system. None of the other compounds tested exhibited any significant effect upon the activity of any of the three enzymes. However, since the coupled enzyme assay previously used by Paglia and Valentine (8) and which requires glutathione reductase, was used in the glutathione peroxidase assay and since the purified commercial

Table I

Effects of Phenolic Compounds and Nicotine on Activity of Three Enzymes from Rat Lung

0.4 mM Effector	Glutathione Peroxidase	Glutathione Reductase	Glucose-6-phosphate Dehydrogenase
None	100	100	100
Caffeic acid	100	102	99
Chlorogenic acid	24	33	13
p-Coumaric acid	102	102	100
Esculetin	12	45	68
Esculin	16	45	49
Ferulic acid	101	100	100
Nicotine	102	102	100
Salicylic acid	102	96	100
Scopoletin	14	37	43
Scopolin	17	55	27
Vanillic acid	100	102	100

glutathione reductase used in the assay system displayed the same pattern of inhibition as the rat lung enzyme, it is not possible at present to determine how much of the observed inhibition can be attributed to the inhibition of glutathione peroxidase. It is speculated that a significant amount of the inhibition is due to glutathione peroxidase because of the excess quantities of glutathione reductase used in the assay; however, quantitative measurements await the use of a direct assay for glutathione peroxidase.

Discussion

Of those phenolic compounds which we have found to have an inhibitory effect on the enzyme systems studied, scopoletin, esculetin, and chlorogenic acid have been previously shown to be present in cigarette smoke. Scopoletin is by far the most abundant of the three in the smoke. In 1958, scopoletin was identified in the mainstream smoke of all 29 different brands of cigarettes tested, some of which had filters (9). The amount of scopoletin present in smoke for each 1 g of tobacco originally present in the smoked cigarette ranged from 10.3 to 27.4 µg. Chlorogenic acid (10) and esculetin (11) have also been identified in trace amounts in cigarette smoke. Scopolin and esculin, which are glucosides of scopoletin and esculetin, respectively, have not been found in cigarette smoke, although they have been reported to be present in tobacco plants (12).

The smoke from cigarettes has been shown by several groups of research workers to inhibit some dehydrogenases. Sato and coworkers (13) have reported that inhibition of succinic dehydrogenase by cigarette smoke was eliminated

by cysteine or glutathione. Lange (14) reported an inhibition of purified rabbit muscle glyceraldehyde-3-phosphate dehydrogenase and yeast alcohol dehydrogenase by cigarette smoke. The observed inhibition of both of these enzymes was partially overcome by prior inhalation of the smoke. Lange suggested that this inhibitory effect of cigarette smoke might be the result of oxidation of SH groups by peroxides which might be present in the smoke. Subsequently (15), peroxides were identified in cigarette smoke.

Using specific effectors and enzyme system assays, we have found that the phenolic lactone scopoletin, a significant component of cigarette smoke, inhibits glutathione reductase, G6P dehydrogenase, and possibly glutathione peroxidase from rat lungs. These enzymes are the very ones that have been postulated by Chow and Tappel (4) to comprise a protective mechanism against lipid peroxidation in mammals.

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